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The effects of adrenergic and cholinergic stimulation on skin gland secretions in the Dwarf African Frog Hymenochirus curtipes

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THE EFFECTS OF ADRENERGIC AND CHOLINERGIC STIMULATION ON SKIN GLAND SECRETIONS IN THE DWARF AFRICAN FROG Hymenochirus curtipes

by

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Abstract

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Many studies have been done on the neural control of serous gland secretion in the skin of frogs and newts. However, no studies have been published on the effects of adrenergic and cholinergic neurotransmitters on the sexually dimorphic breeding glands of male frogs. The present study examined the effects of neurotransmitters on the serous and breeding glands of Hymenochirus curtipes. Explants of dorsal skin and postaxial skin (containing whole breeding glands) were incubated in vitro with epinephrine, norepinephrine or acetylcholine for 30 minutes. The explants were then preserved and examined histologically for signs of secretion. The area and perimeter of the serous and breeding glands were measured before (control groups) and after the treatments.

Epinephrine and norepinephrine treatments decreased the overall area of the serous gland. However, acetylcholine had no effect on serous gland size. The effect of epinephrine on serous gland

area was partially blocked by the adrenergic antagonist phenoxybenzamine. Measurement of individual lobes and whole breeding glands after 30 minutes of treatment (with epinephrine, norepinephrine, and acetylcholine) showed no significant change in area or perimeter. This experiment confirmed earlier studies demonstrating that adrenergic and not cholinergic stimulation can affect the secretion of serous glands in frogs. However, specific antagonists can mask these effects. In contrast, breeding gland secretion showed no variation in overall area or individual lobe size. Thus, breeding gland secretion is not regulated by adrenergic or cholinergic systems.

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INTRODUCTION

The amphibian skin is unique among terrestrial vertebrates in that it has the greatest abundance of multicellular exocrine glands. Amphibian skin is water permeable and is often colorful. The skin performs many functions. It protects against abrasion and pathogens, serves as a respiratory membrane, helps reduce water-loss, and helps regulate body temperature. In addition, poisons in the skin help protect the animal against predators. It is believed that skin secretions play a major role in many of the these functions.

The exocrine glands imbedded in the dermis of amphibians have received considerable attention from morphologists (e.g., Hoffman and Dent, 1977; Holmes and Balls, 1978; Thomas, Tsang and Light, 1993). Earlier workers believed only one type of gland to be present in amphibians (Muhse, 1909). It is now recognized that amphibians typically have two types of glands: mucous and serous (=poison or granular). Some amphibians may also have as many as two or three more types: seromucous, lipid, and breeding glands (Duellman and Trueb, 1986; Thomas et al., 1993).

Serous Glands

Serous glands (see Fig. 1A) are so named because they have a serous appearance when stained with plasma dyes (Porter, 1972).

The glands are often concentrated in strategic areas, such as the parotoid glands behind the heads of toads and some frogs or the granular ridges along the backs of salamanders. Serous glands produce milky secretions that are often toxic to predators. In some frogs, serous gland secretions contain alkaloid substances which resemble digitalis in action. These venoms may be sufficiently potent to kill large vertebrates by increasing the tonicity of the heart, weakening respiration, and causing general muscular paralysis (Porter, 1972). Serous glands have been shown to have one or two kinds of myoepithelial cells (MECs)(Quay, 1972). These MECs have also been shown to be connected by desmosomes (Fujikura, Kurabuchi, and Inoue, 1988). Serous secretions are secreted through excretory ducts which open onto the surface of the skin. The duct is made up of two different layers of cells.

The serous glands in the African clawed toad Xenopus laevis consists of a syncytial secretory compartment surrounded by a single layer of MECs. The MECs of the serous glands of X. laevis posses alpha and beta-adrenorecepters. Stimulation of the MECs by alpha-adrenergic agonists results in expulsion of the contents of the gland, but contraction can be blocked or opposed by a number of agents (Holmes and Balls, 1978).

Another study using X. laevis hypothesized that high concentrations of peptides such as caerulein (similar to gastrin) may be a serous gland secretory product (Dockray and Hopkins, 1975). This study demonstrated that caerulein in the skin is contained in

secretion granules within the serous glands and that its release can be specifically evoked by adrenergic stimulation.

The serous glands of the leopard frog, Rana pipiens, are also stimulated by alpha-adrenergic agents. For example, agonists such as epinephrine, norepinephrine, and phenylephrine (in order of effectiveness) can cause stimulation of serous glands (Holmes and Balls, 1978). Alpha-adrenoreceptor agonists induce contraction of MECs and the discharge of serous contents. However, alpha-adrenoreceptor antagonists and the stimulation of beta-adrenoreceptors will block the serous secretion.

Unmyelinated nerves have been shown to lead under the surface of the serous glands of the common toad Bufo bufo and the common frog Rana temporaria (Fox, 1986). The serous glands in the common frog receive adrenergic stimulation. Nerve terminals were found near the basement membrane but never within the MECs of mucous glands (see Fig. 1B). However, they were located between the MECs and in direct contact with the secretory cells of the serous glands. The diffusion of released neurotransmitters from these nerve endings to the MEC plasma membrane causes the contraction and expulsion of the serous contents of the gland (Holmes and Balls, 1978). The presence of nerve terminals within the secretory epithelium may influence serous gland secretion (Fox, 1986).

These studies support the view that, in frogs, the discharge of the serous glands is under direct nervous control, and provide a morphological basis for the interpretation of previous studies that secretion by dermal glands in X. laevis is stimulated by epinephrine.

In contrast to serous glands in anurans, which are stimulated by alpha-adrenergic agonists, the serous glands of the red-spotted newt (Notopthalmus viridescens) are stimulated by acetylcholine (Hoffman and Dent, 1977). This same study also showed that the serous secretion of the newt can be blocked by atropine.

Mucous Glands

The second type of dermal gland is the mucous gland (Fig. 1B). The typical mucous gland lacks a distinct myoepithelium. In comparison, mucous and serous glands are surrounded by MECs in anurans. Typically, MECs in a single layer join one another by desmosomes (Fox, 1986).

Mucous glands are more numerous and widely distributed throughout the integument than are serous glands. Mucous glands are generally smaller than serous glands. The numbers of mucous and serous glands vary throughout the body. Generally, mucous glands are more abundant in the dorsal skin. The mucous cells of the mucous glands are greatly swollen with mucus. The ducts are narrow, and lined with a layer of small flattened epithelial cells. The body of the gland is lined with epithelium which forms the mucus. After formation, the mucus is discharged into the lumen of the gland. The appearance of the secreting epithelium varies greatly in different glands (Holmes, 1927).

During secretion these mucous granules swell, and change into a transparent substance which is discharged into the central cavity. The changes in the epithelial cell structure will eventually transform

transform these apocrine cells into mucus. The changes of the cell structure is also attributed to contraction of the gland itself (Holmes, 1927).

Previous investigators have stated that only one type of mucous gland exist (Neuwirth, Daly, Myers and Tice, 1979). However, further studies (Mills, Rick, Doerge and Thurau, 1982) showed differences in the morphology of the mucous glands Electron-microprobe studies (spectral fingerprint of the mucus and freeze-dried sections) of the skin gland cells have shown two distinct types of mucous glands: mucous and seromucous glands (Mills and Prum, 1984). The possibility exists that the glands are in developmentally or biochemically different states of the same gland. The study done by Mills and Prum brings up a legitimate possibility that mucous and serous glands are related. Cells within both the mucous and seromucous glands respond to beta-adrenergic stimulation as determined by changes in the intracellular ions (Mills and Prum, 1984).

In another study, Skoglund and Sjoberg (1977b) noted that some mucous glands were in a refractory phase after intense nerve stimulation. However, eventually every observed gland did contract. Thus, it seems mucous and seromucous glands have the capability to secrete by two distinct mechanisms (Mills and Prum, 1984). However, previous studies on the mucous glands of the red-spotted newt showed no discharge in response to either acetylcholine or adrenergic agents (Hoffman and Dent, 1977).

Gland Development

Contrary views exist on the development of mucous and serous glands. For example, Bovbjerg (1963) indicated that in Rana pipiens, the two types of glands and secretory cells develop independently without intermediate or transitional types. However, Bovbjerg also concluded that the serous glands differentiate before the mucous glands. Other studies in salamanders have shown intermediate stages between mucous to serous cells (see Duellman and Trueb, 1986, for review). Another study concluded that the serous glands are shared primitive characters among amphibians and their original function probably was not poison synthesis. Nevertheless, the glands were thought to be a preadaptation for producing the diverse toxins that evolved separately in some groups of amphibians (Neuwirth et al., 1979).

Lipid Glands

Lipid glands (wax gland) exist in the integuments of a few anurans. The lipid glands are slightly larger than serous glands. They are located basally in the stratum corneum. Unlike the mucous glands, the lipid glands have a distinct myoepithelium (Blaylock, Ruibal and Platt-Aloia, 1976). Lipid-secreting glands are found in abundance in the skin of the uricotelic frogs (e.g., the leaf frog Phyllomedusa sauvagii). These frogs live in semiarid environments. The waxy secretion is used to retard water loss through the skin and is spread over the skin surface by a grooming action of the limbs. These frogs appear able to sense when the impermeability of the

skin surface has been disrupted and they make appropriate secretory and wiping responses. Following these grooming movements, the skin assumes a glossy, dry appearance and the rate of water loss drops rapidly (Stebbins and Cohen, 1995).

Characteristics of Courtship

Successful propagation of anuran genes depends on the location of potential mates, stimulation of mates, selection of breeding site, fertilization of the eggs, and development of the eggs and young (Duellman and Trueb, 1986). Generally, during courtship males are the more aggressive sex, and their courtship activities depend on female response. In addition to the reproductive organs and their associated tracts, external sexual differences exist in most amphibians, including size, glandular development, skin texture, dermal ornamentation, vocal sacs, and coloration (Duellman and Trueb, 1986). Some differences persist throughout adult life, but others develop in response to gonadotropic hormones. Some structures are used in courtship and others, for holding the pair in an embrace during mating.

Secondary Sexual Characteristics

Several secondary sexual characters exists in anurans. The first type is spines and tusks. The best case of spines is found in the gladiator frogs (Hyla boans) in which mates defend their nests by grappling. Sharp tusks of the lower jaw occur in both sexes of several kinds of frogs, especially carnivorous types such as

Ceratobatrachus, Hemiphractus, and Pyxicephalus (Duellman and Trueb, 1986). The second type is nuptial excrescences. The nuptial excrescences consist of modified dermal and epidermal tissues. In some frogs, they extend distally on the thumb and also may be present on the median or dorsal surfaces of the second and third fingers and or on the ventromedial surface of the forearm. Most frogs which have nuptial excrescences undergo amplexus (when the male frog grasps the female so that he is dorsal to her) in water (Duellman and Trueb, 1986).

The third type of secondary sexual characteristic is the development of "breeding" glands in male anurans. These glands may appear in a variety of places on the anuran. For example, breeding glands have been found in the pectoral, abdominal, femoral, postaxial, ventrolateral, and humeral regions of anurans (Duellman and Trueb, 1986). The function of these glands is unknown. However, since most are in contact with the female during amplexus, the secretions from these glands may have a stimulating effect on ovulation. Abdominal breeding glands are present in many microhylids (narrow-mouthed toads) that are excessively round in body shape. These glands secrete an adhesive substance that helps the male maintain amplexus (Conaway and Metter, 1967). To date, there have been no published reports investigating the neurotransmitters involved in stimulating breeding gland secretion. In the dwarf African frog, H. curtipes, males possess a distinctive breeding gland located in the postaxial skin, in addition to serous and mucous glands.

The present study was conducted to show the effects of neurotransmitters on H. curtipes skin explants and whole breeding glands. Epinephrine and norepinephrine were used as adrenergic neurotransmitters to see if these agents would affect the serous and breeding glands. Acetylcholine was also used on the explants and breeding glands to see if cholinergic stimulation was possible. Phenoxybenzamine hydrochloride and atropine were used as blockers of epinephrine and acetylcholine, respectively. Similar studies have been done on serous glands of X. laevis (Benson and Hadley, 1969; Hoffman and Dent, 1977). However, no published work has been done on H. curtipes. The breeding glands were subjected to the same drug treatments as the skin explants. No published pharmacological data has been collected on breeding glands itself.

Thus, the objective of this study was to substantiate earlier studies (from other frogs) that epinephrine and norepinephrine stimulate serous gland secretion in H. curtipes. In turn, this lead to the hypothesis that the effects of adrenergic or cholinergic stimulation may stimulate the breeding glands of H. curtipes.

MATERIALS AND METHODS

Animals

A total of 48 mature male dwarf African clawed frogs (Hymenchirus curtipes) were obtained from a commercial breeding colony (Blue Lobster Farms, Madera, CA). The frogs were housed in a plastic rectangular container (20L). The frogs were kept in filtered tap water. The frogs were fed with live tubifex worms.

Chemicals

Three neurotransmitters were used in this study. Epinephrine, norepinephrine and acetylcholine chloride were obtained from the Sigma Chemical Company (St. Louis, MO). Two blockers (antagonists) were used in this study. Epinephrine was blocked by phenoxybenzamine hydrochloride, obtained from Spectrum (Gardena, CA) and acetylcholine was blocked by atropine (Sigma Chemical Company, St. Louis, MO). The concentration of all treatment groups was at 1 mM in amphibian Ringers solution. Note: Atropine and phenoxybenazamine (blockers for acetylcholine and epinephrine respectively) were each dissolved in ethanol. Atropine was dissolved in ~ 5 ml of ethanol (final concentration of ethanol was 1%) . However, phenoxybenzamine was dissolved in ~5 ml of ethanol

(final concentration of ethanol was 1%) and then placed on a hot plate for approximately 1/2 hour (gently warming).

Tissue Collection

All animals were anesthetized on ice and then decapitated. Samples of dorsal skin were taken dorsomedially. The skin samples were cut into two halves (approx. 25mm^2), then placed in test tubes filled with approximately 3 ml of ice cold amphibian Ringer's solution. The breeding glands, attached to small sections of skin (approx. 20-25mm^2), were excised from the postaxial area behind the forearms. The breeding glands, one from each side of the body, were placed in ice cold Ringer's solution.

A total of six treatment groups were studied. Within these six treatment groups there were eight frogs per treatment group. For each frog four tissue samples were excised. For each set there existed a left and right pair. These were individually matched within frogs, but not necessarily matched with left and right sides. The skin and breeding samples were designated by the following abbreviations: D-L/D-R for left and right dorsal skin samples respectively, B-L/B-R for the left and right breeding glands respectively. The left side tissues in all treatment groups were used as internal controls. In addition, an external control group (skin explants and breeding glands which did not receive treatments and were fixed following excision) was also utilized to backup the internal controls.

Experimental Procedure

All in vitro control tissues (tissues designated as "left-side" or "untreated") were rinsed twice for 30 minutes in room temperature Ringer's. The control tissues and glands were then placed in fixative (A.F.A.=formalin 10%, ethanol 48%, acetic acid 2%, and distilled water 40%) overnight.

For treated tissues (designated as "right-side") the procedure followed one of two sequences. First, the epinephrine, norepinephrine, and acetylcholine treatment groups were rinsed once in room temperature Ringer's for 30 minutes. The Ringer's was discarded, then each of these treatment groups received their respective adrenergic or cholinergic drug. The adrenergic and cholinergic treatments were allowed to incubate in vitro for 30 minutes. The tissues were removed from the treatment solutions and transferrred to fixative overnight.

The second sequence of treatments was carried out slightly differently. The epinephrine/phenoxybenzamine treatment group was pretreated with the Ringer's/phenoxybenzamine for 30 minutes instead of the initial rinse with room temperature Ringer's. After 30 minutes the Ringer's/phenoxybenzamine was discarded and the epinephrine/phenoxybenzamine was added and allowed to incubate in vitro for 30 minutes. The tissues and glands were then placed in fix overnight. The acetylcholine/atropine group was also pretreated with Ringer's/atropine and allowed to incubate for 30 minutes. The Ringer's/atropine was then discarded. Acetylcholine/atropine was then added and allowed to incubate for 30 minutes. The

acetylcholine/atropine was then discarded. The tissues were then placed into fixative overnight.

Histology

Fixed tissues were dehydrated using a graded series of ethanol (35-50-70-95-100%). The tissues were then cleared in Hemo-De (Fisher Scientific) and embedded in Paraplast. The tissues were sectioned at 10μm on a Spencer 820 micortome with steel knives. Next, the paraffin sections were deparaffinized and hydrated through a graded series of ethanol. The tissues were then stained with Harris' Hematoxylin (Fisher Scientific) and eosin (0.5% solution in deionized water) using standard methods (Galigher and Kozloff, 1971).

Microscopy and Data Analysis

Serous glands were examined using a Bausch and Lomb compound microscope (Balplan) and were photographed using a 40X objective and a 10x eyepiece. Video frames ("photographs") were obtained with a Video Labs video camera (Video Plus T230 program). The camera was coupled to the eyepiece with a plastic collar. From each photo of a serous gland, the glands cross-sectional surface area was measured using Sigma Scan (Jandel Scientific). The area of each serous gland was measured five times, and averaged, then these average gland sizes were summed and averaged to calculate a mean gland area for each treatment group.

The breeding glands were examined using an Olympus dissecting scope and were photographed at 4X (thorugh a 10x eyepiece). In addition, individual lobes of the breeding glands were photographed at 40X using a Bausch and Lomb compound microscope. The breeding glands and lobes were photographed using a Video labs video camera (Video Plus T230 program). From each photo of the breeding gland and breeding gland lobe, the glands cross-sectional surface area was measured using Sigma Scan (Jandel Scientific). The breeding glands and individual lobes were each measured five times and then averaged as described above. Sigma Scan was utilized for measuring the breeding glands and individual lobes .

Breeding glands were measured for area and perimeter. First, 8 whole breeding glands were measured for each treatment group. The averages were then used to compare the different treatment groups. From each breeding gland, five individual lobes were measured (five times each for area and perimeter) to see if changes in gland size occurred internally. The area and perimeter of the glands were also compared to their internal and external controls.

The average areas and perimeters of treated glands were compared against matching measurements for untreated glands, then expressed as a percent of untreated gland size.

Epithelial height was measured five times, and averaged, then these average epithelial heights were summed and averaged to calculate a mean epithelial height for each treatment group.

Statistics

The data were analyzed statistically. A t-test (two-sample assuming unequal variances) was used to compare the left side (control) with the right side (treatment) of all treatment groups. The level p (observed significance level)$\geq$.05 was used to state that the data is not statistically different than the other data that is being compared.

RESULTS

Morphological Characteristics of Serous Glands

Serous glands are composed of a secretory compartment, a duct and an intermediate region connecting them. The secretory compartment consists of syncytial sac surrounded by a myoepithelial cell layer (Fig. 2). The secretory granules which occupy the majority of the syncytium were elipsoidal in shape. The secretory granules had a tendency to displace to the basal region of the secretory compartment. The excretory duct of the serous gland is opened throughout its length onto the surface of the skin. On occasion some secretory materials were found in the ductal lumen.

After treatment with epinephrine and norepinephrine the serous glands were noticeably smaller. In some cases the connective tissue surrounding the serous glands was slightly detached from the myoepithilium. Also, shrinkage of the serous gland was readily apparent.

Morphological Characteristics of Breeding Glands

Breeding glands are composed of many lobes. These individual lobes are grouped together by sheaths (Fig. 3c). It was also observed that the lobes contained by the sheaths collected into a central draining duct which lead to the skin. Interestingly, several ducts

could be seen leading to the skin (Fig. 3d). The ducts that were observed could be traced back in origin to the location of the sheaths surrounding the lobes of the breeding glands. This observation had not been reported previously.

After the treatments open cavities were found in some of the acetylcholine treated breeding glands (Fig. 3B). The size of these cavities varied in shape and size. In most instances these cavities could be traced to a duct leading to the skin. This observation had not been reported previously.

Changes in Skin Glands under the Experimental Conditions

Serous Glands

The epinephrine and norepinephrine treated serous glands showed a reduction in area and perimeter following the treatment (Table 2, Figs. 4-6). However, acetylcholine treated glands did not show any significant reduction or increase in area or perimeter. The effects of epinephrine were inhibited by the alpha-adrenergic blocker phenoxybenzamine. Phenoxybenzamine was found to reduce the response of the epinephrine treated serous glands. The epinephrine/phenoxybenzamine treatment group showed a slight decrease of overall area and perimeter (Fig. 5 and 6). However, the epinephrine/phenoxybenzamine results were not statistically significant from the control group. Lastly, the acetylcholine/atropine treatment group changed very little in area and perimeter compared to acetylcholine alone(Fig. 4B and Table 2).

Thus, adrenergic stimulation caused the stimulation of serous glands. In turn this caused the cross-sectional area and perimeter to decrease. Cholinergic stimulation did not occur since acetylcholine by itself did not cause the serous glands to discharge. While there were notable differences in gland size following adrenergic stimulation, and not after cholinergic stimulation, none of the treatments resulted in changes that were statistically different from untreated glands.

Breeding Glands

The same treatment groups and neurochemical concentrations used in the serous gland portion of these experiments were used in the breeding gland portion of this study. The results showed that neither epinephrine, norepinephrine nor acetylcholine had any significant effect on the overall area or perimeter of the breeding glands (Table 3, Fig. 7 and 8). Likewise, the epinephrine/ phenoxybenzamine and acetylcholine/atropine results for the whole breeding gland showed no significant change in area or perimeter as compared to epinephrine and acetylcholine respectively.

The individual lobes of the breeding glands were examined to determine if the treatments were affecting the lobes and not the whole breeding gland. The results of area and perimeter measurements of the lobes (Table 4, Fig. 9-10) correlated with the size of whole breeding glands (Table 3, 4, Fig. 7-8).

Lastly, the epithelial cell layer of the individual lobes were measured (Table 5 and Fig. 11). This was done to see if the size of the cells within the lobes changed after the treatments. The distance

from the apical cell membrane of the lobe to the basal membrane was unchanged after the treatments (Table 5, Fig. 11). Thus, neither the adrenergic nor the cholinergic chemicals had any affect on the area or perimeter measurements of the breeding glands.

DISCUSSION

Serous Glands

Most morphological and pharmacological studies of the skin glands of frogs have dealt with the serous glands. This interest relates to the presence of potent pharmacologically active components such as vasoactive peptides and steroidal alkaloids in frog skin (Neuwirth et al., 1979). Dockray and Hopkins (1975) correlated release of caerulein (gastrin-like peptide) with discharge of serous glands in X. laevis. It is has been found that these peptides are contained within the serous gland (Neuwirth et al.)

The serous gland has been described as surrounded by smooth muscle (Noble and Noble, 1944) or myoepithelium (Dockray and Hopkins, 1975). It should be noted that the smooth muscle cell layer is derived from the same layer as the myoepithelial cells of the secretory membrane. Thus, the two names for the surrounding layers may be one and the same. It is clear that in the serous glands these cells respond to alpha-adrenergic stimulation or nerve stimulation by contraction, and this results in the expulsion of the serous contents (Benson and Hadley, 1969; Hoffman and Dent, 1969; Dockray and Hopkin, 1975).

The present study has shown that epinephrine and norepinephrine (at 1 mM) can cause the stimulation of serous glands

of H. curtipes in vitro. These results are consistent with earlier studies in other frogs (Benson and Hadley, 1969; Hoffman and Dent, 1977; Dockray and Hopkin, 1975). The effect of cholinergic stimulation in this study proved to be negative in serous glands. No significant change in area or perimeter was detected after the acetylcholine treatment. In contrast, a similiar study (Hoffman and Dent, 1977) on the red-spotted newt (N. viridescens) showed serous gland discharge after exposure to acetylcholine (at 1 mM).

Phenoxybenzamine was shown in H. curtipes to reduce the effects of epinephrine in the serous glands. No significant changes in area and perimeter were detected. The study done by Hoffman and Dent (1977) used ergotamine to block the effects of epinephrine. Findings in that study correlate with the present findings. The present study also used atropine to block the effects of acetylcholine. Acetylcholine by itself did not show any stimulation. Thus, when atropine was added one would expect the same result. Indeed, that was the case in this experiment. No significant change in area and perimeter was detected. This particular finding is also substantiated in the study done by Hoffman and Dent (1977).

The innervation of mucous and serous glands is very different. Studies (Falck, Thieme and Torp, 1962, Sjoberg and Flock, 1976) have indicated that the frog skin glands receive exclusively adrenergic innervation. No cholinergic innervation has been found in frog skin glands. In the mucous gland the nerve terminals are located outside the gland parenchyma (see Fig. 1B). In contrast, the serous gland's nerve terminals are present within the parenchyma in close

conjunction with muscle cells and with the secretory epithelium (see Fig. 1A). Findings in X. laevis have shown that nerve endings are present in the secretory compartment of serous glands (Dockray and Hopkins, 1975). Also, Sjoberg and Flock (1976) reported a similar finding in the serous gland of R. temporaria and R. esculenta.

Since nerve terminals are present within the secretory epithelium of the serous gland, a direct neuronal control of the secretion is possible. The comparatively rich supply of nerves to these cells, with terminals in direct contact with their plasma membrane, suggests that they may be essential for the rapid and synchronous stimulation of this muscle layer (Sjoberg and Flock, 1976). The rapid and synchronous stimulation of the muscle layer may be necessary in order for the secretion products to be expelled.

Previous studies have shown neural influences may be exerted in the skin glands differently. Some glands are innerevated on the inside of the secretory compartment while other glands are only innervated outside the secretory compartment. The excretion of the contents of serous glands may occur mainly due to contraction of the myoepithelial cells. Histological signs of contraction of the myoepithelial cells were evident in the report by Holmes and Balls (1978). However, a more recent study (Fujikura et al., 1988) showed that there were also nerve endings resting on the surfaces of the secretory cells and occasionally far from the surface of the myoepithelial cells. Therefore it is important to study the receptor sites for adrenergic stimulation not only through the surface of the myoepithelial cells but also through the secretory cells. Studying the

secretory cells may bring to light the cellular mechanism by which the contents of the serous gland are expelled upon adrenergic stimulation.

The secretory process in the serous glands warrants further and more detailed study. It is of interest in that the release of secretory product does not involve exocytosis (Dockray and Hopkins, 1975), and consequently, the collection and assay of intact secretory granule has been possible This feature is particulary advantageous in the use of this system as a model in investigations of the biosynthesis and secretions of a small peptide. Moreover, for studies of biosynthesis alone the system also offers the opportunity of depleting the glands and thus synchronizing the processes of restoration and the replenishment of the stores of secretory product.

Breeding Glands

Breeding males of many species of anurans may exhibit glandular areas that appear to relate to reproductive activity. Depending on the species, swollen glandular areas may be present on ventral surfaces in various locations (e.g., on the throat, chest, abdomen, and base of the forearms and hindlimbs; Duellman and Trueb, 1986).

Abdominal glands are present in many microhylids that are excessively rotund-bodied (e.g., Breviceps, Gastrphryne, Kaloula). These glands secrete an adhesive substance that helps the male maintain amplexus (Conway and Metter, 1967).

Thomas et al. (1993) recognized sexually dimorphic skin glands (SDSG) in anurans as a unique gland type, set apart from mucous and serous glands. The function of SDSG in anurans appears to be unknown. However, the secretions of these SDSG may enhance the male's grip during amplexus or may release chemical signals during the mating season (Duellman and Trueb, 1986). The secretions of these SDSG may have more than one function.

The present study has found that the breeding glands of H. curtipes are not affected by adrenergic or cholinergic chemicals. Therefore, one could conclude that the breeding glands are stimulated on a completely different level. These breeding glands are more likely stimulated in response to reproductive activities. There is no direct evidence for the hypothesis that the breeding glands release chemical signals associated with reproduction. It is interesting that anuran breeding glands share anatomical and chemical features with the hedonic glands of caudate amphibians. Hedonic glands are also multicellular, alveolar glands filled with granular secretions of a mucoprotein nature (Thomas et al., 1993). These glands function during mating and can secrete pheromones associated with reproduction. The anatomical and chemical similarities of anuran breeding glands and caudate hedonic glands may reflect a common evolutionary pathway (Thomas et al., 1993). An earlier study done by Pool, Dent and Kemphues (1977) showed that incubation of newt skin explants possessing hedonic glands in solutions of acetylcholine decreased the luminal diameters of the

hedonic glands. Atropine inhibited the acetylcholine activity. However, epinephrine had no effect (for review see Fox, 1986).

Recently gonadotropin-releasing hormone (GnRH) was found to increase the overall area of the breeding glands in H. curtipes (Vahamaki and Thomas, 1997). The possibility exists that the responses to GnRH may occur in parallel with breeding gland secretion. GnRH may cause the breeding gland to secrete indirectly through the pituitary-testis axis system. This is a significant finding given the results of this study that breeding glands are insensitive to adrenergic and cholinergic stimulation.

In conclusion, this experiment has confirmed earlier studies that adrenergic and not cholinergic stimulation can affect the secretion of serous glands. However, specific antagonists can mask these effects. In contrast, breeding glands showed no variation in overall area or individual lobe size. Thus, breeding gland secretion is not regulated by adrenergic or cholinergic systems. Further study should be done on these breeding glands to elucidate the role and stimulation of these so called "breeding glands."

Table 1. Chemicals Affecting Release of Dermal Serous Glands in Xenopus laevis

(Adapted from Benson and Hadley, 1969 and Holmes and Balls, 1978)

EFFECT	FACTOR
Stimulation of release	Alpha-Adrenoreceptor agonists: (epinephrine, norepinephrine, phenylephrine)
	High K+
	Calcium availability
Opposition of stimulation	Alpha-Adrenoreceptor antagonists: (phenoxybenzamine, phentolamine, thymoxamine)
	Beta-Adrenoreceptor agonists: (isoprenaline, salbutamol, epinephrine)
	Theophylline
	Antihypertensive agent(Diazoxide)
	High calcium
	Atropine
Blockade of Beta-adrenergic opposition to stimulation	Beta-Adrenoreceptor antagonists (timolol, sotalol)
No stimulation or inhibition	Acetylcholine
	Angiotensin II
	Clonidine
	Histamine

Table 2. Average Serous Gland Cross Sectional Area and Perimeter in Hymenochirus curtipes Under Various Treatment Conditions (mean +/- standard error)

		AREA (μm^2)	PERIMETER (μm)
Control	LEFT	14,193 +/-1,310 [8]	485 +/-27
	RIGHT	15,116 +/-1,988 [8]	511 +/-41
		p= .35	p= .44
Epinephrine	LEFT	9,552 +/-1,756 [8]	413 +/-32
	RIGHT	6,833 +/-1,127 [7]	348 +/-31
		p= .11	p= .19
Norepinephrine	LEFT	10,373 +/-1,613 [8]	406 +/-29
	RIGHT	8,248 +/-1,530 [8]	358 +/-33
		p= .18	p= .15
Acetylcholine	LEFT	13,679 +/-519 [5]	464 +/-6
	RIGHT	13,459 +/-1,007 [6]	449 +/-26
		p= .43	p= .31
Epinephrine/ Phenoxy-benzamine	LEFT	6,834 +/-1,135 [8]	321 +/-28
	RIGHT	5,424 +/-891 [6]	292 +/-27
		p= .17	p= .23
Acetylcholine/ Atropine	LEFT	9,707 +/-3,180 [6]	397 +/-53
	RIGHT	9,391 +/-1,685 [7]	382 +/-35
		p= .47	p=.41

[ ]: Number of frogs examined
p= Significance level

Table 3. Average Breeding Gland Cross Sectional Area and Perimeter in Hymenochirus curtipes Under Various Treatment Conditions (mean +/- standard error)

		AREA (μm^2)	PERIMETER (μm)
Control	LEFT	136,724 +/-12,650 [8]	5,325 +/-276
	RIGHT	152,858 +/-25,622 [7]	5,785 +/-644
		p= .29	p= .27
Epinephrine	LEFT	107,387 +/-9,189 [8]	4,945 +/-230
	RIGHT	107,088 +/-10,776 [7]	4,796 +/-253
		p= .49	p= .33
Norepinephrine	LEFT	131,123 +/-22,920 [6]	5,325 +/-506
	RIGHT	186,841 +/-36,754 [8]	6,348 +/-644
		p= .11	p= .12
Acetylcholine	LEFT	142,807 +/-22,552 [8]	5,647 +/-483
	RIGHT	166,233 +/-22,644 [6]	6,153 +/-414
		p= .24	p= .23
Epinephrine/ Phenoxy-benzamine	LEFT	171,040 +/-18,952 [7]	6,314 +/-380
	RIGHT	154,112 +/-19,654 [6]	5,808 +/-437
		p= .27	p= .20
Acetylcholine/ Atropine	LEFT	164,519 +/-20,252 [8]	6,095 +/-449
	RIGHT	149,742 +/-21,574 [8]	5,934 +/-483
		p= .07	p= .40

[ ]: Number of frogs examined
p= Significance level

Table 4. Average Breeding Gland Lobe Cross Sectional Area and Perimeter in Hymenochirus curtipes Under Various Treatment Conditions (mean +/- standard error)

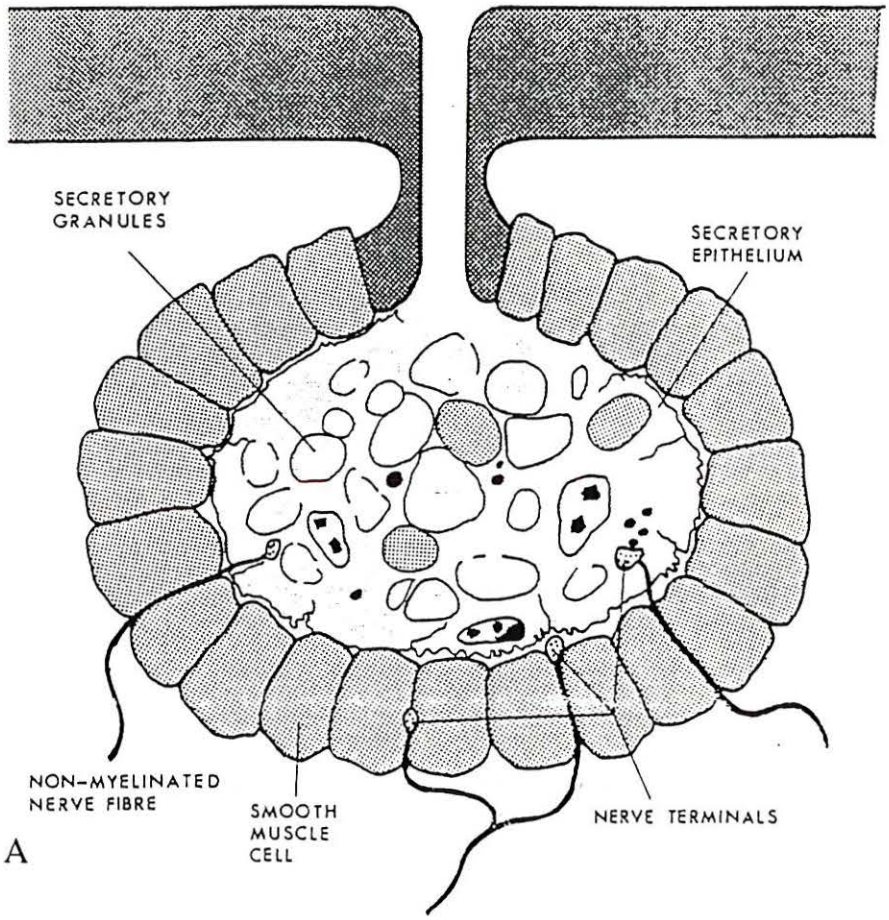
		AREA (μm^2)	PERIMETER (μm)
Control	LEFT	2,322 +/- 336 [8]	193 +/-15
	RIGHT	2,097 +/- 91 [8]	180 +/- 5
		p= .27	p= .25
Epinephrine	LEFT	3,013 +/-561 [8]	200 +/-9
	RIGHT	2,388 +/-171 [7]	195 +/-6
		p= .16	p= .32
Norepinephrine	LEFT	2,388 +/-238 [6]	196 +/-9
	RIGHT	2,965 +/-644 [8]	186 +/-7
		p= .21	p= .22
Acetylcholine	LEFT	1,631 +/-139 [8]	157 +/-6
	RIGHT	1,741 +/-149 [6]	159 +/-7
		p= .30	p= .42
Epinephrine/ Phenoxy-benzamine	LEFT	3,119 +/-226 [7]	321 +/-28
	RIGHT	3,013 +/-351 [6]	292 +/-27
		p= .40	p= .23
Acetylcholine/ Atropine	LEFT	3,375 +/-170 [8]	233 +/-6
	RIGHT	3,523 +/-224 [8]	237 +/-7
		p= .30	p= .33

[ ]: Number of frogs examined
p= Significance level

Table 5. Average Epithelial Cell Layer of Breeding Gland Lobes in Hymenochirus curtipes Under Various Treatment Conditions (mean +/- standard error)

		HEIGHT (μm)
Control	LEFT	22 +/- .58 [8]
	RIGHT	22 +/- .96 [7]
		p= .16
Epinephrine	LEFT	26 +/- 1.06 [8]
	RIGHT	25 +/- 1.09 [7]
		p= .13
Norepinephrine	LEFT	27 +/- 1.03 [6]
	RIGHT	25 +/- 1.04 [8]
		p= .11
Acetylcholine	LEFT	21 +/- 1.03 [8]
	RIGHT	22 +/- 1.29 [6]
		p= .27
Epinephrine/ Phenoxy-benazamine	LEFT	28 +/- 1.15 [7]
	RIGHT	29 +/- 1.14 [6]
		p= .21
Acetylcholine/ Atropine	LEFT	30 +/- .65 [8]
	RIGHT	30 +/- 1.85 [8]
		p= .5

[ ]: Number of frogs examined
p= Significance level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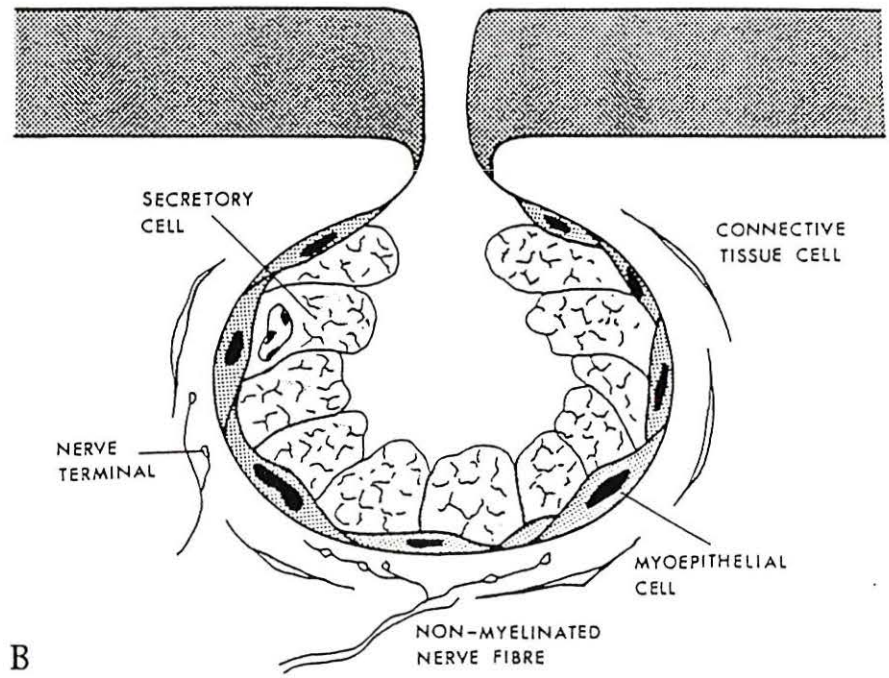
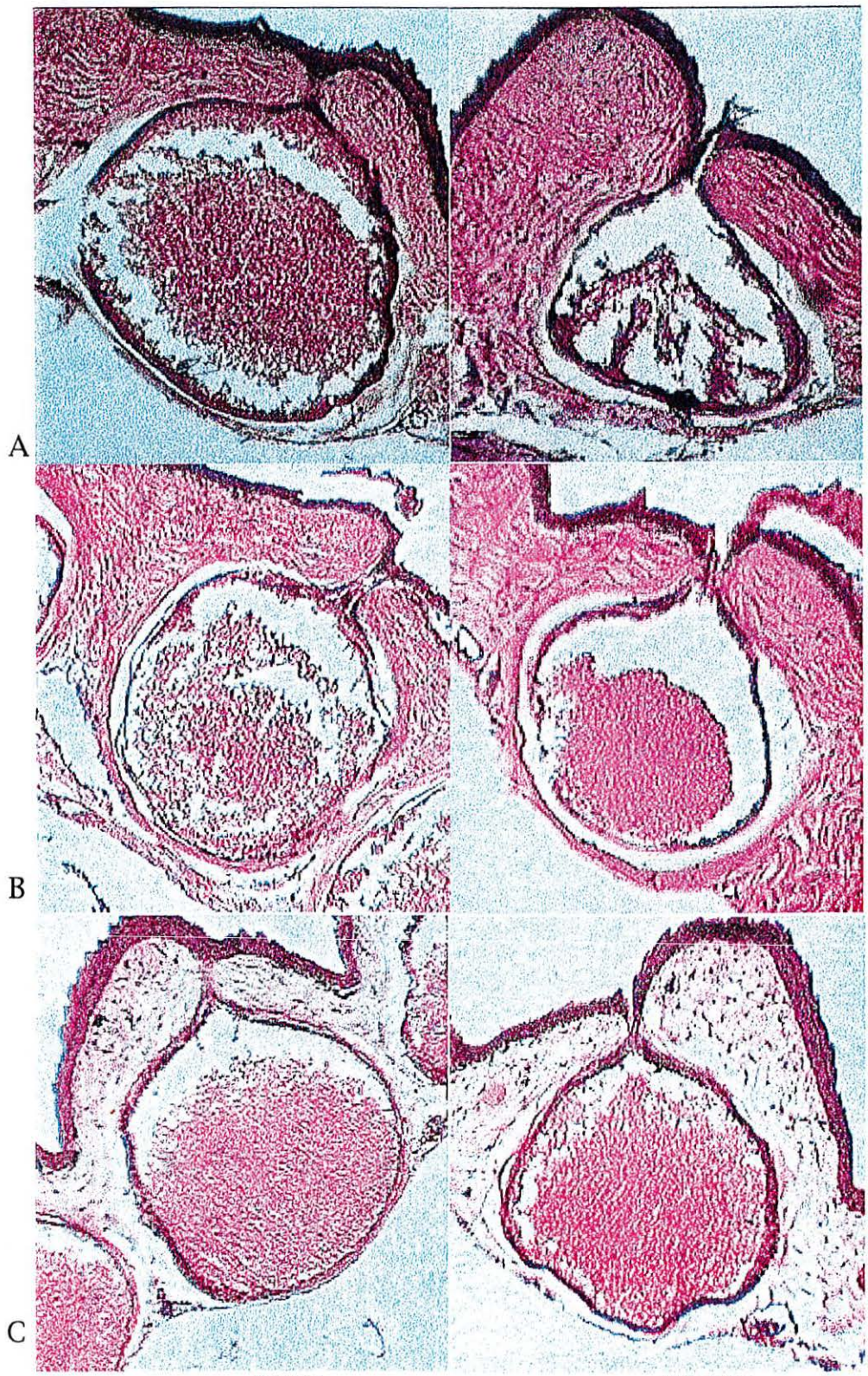
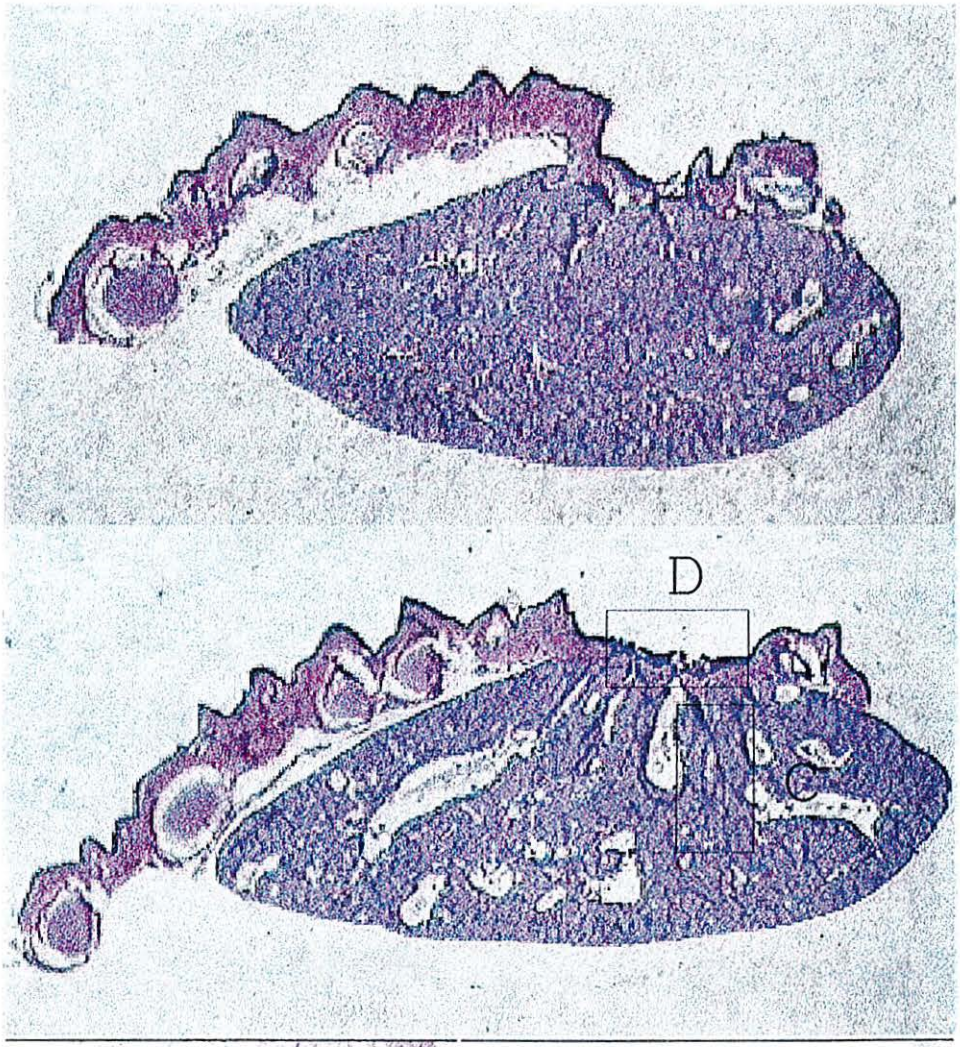
SECRETORY EPITHELIUM
NON-MYELINATED NERVE FIBRE
SMOOTH MUSCLE CELL
NERVE TERMINALS
A


SECRETORY CELL
CONNECTIVE TISSUE CELL
NERVE TERMINAL
MYOEPITHELIAL CELL
NON-MYELINATED NERVE FIBRE
B


A
B
C
LEFT
RIGHT

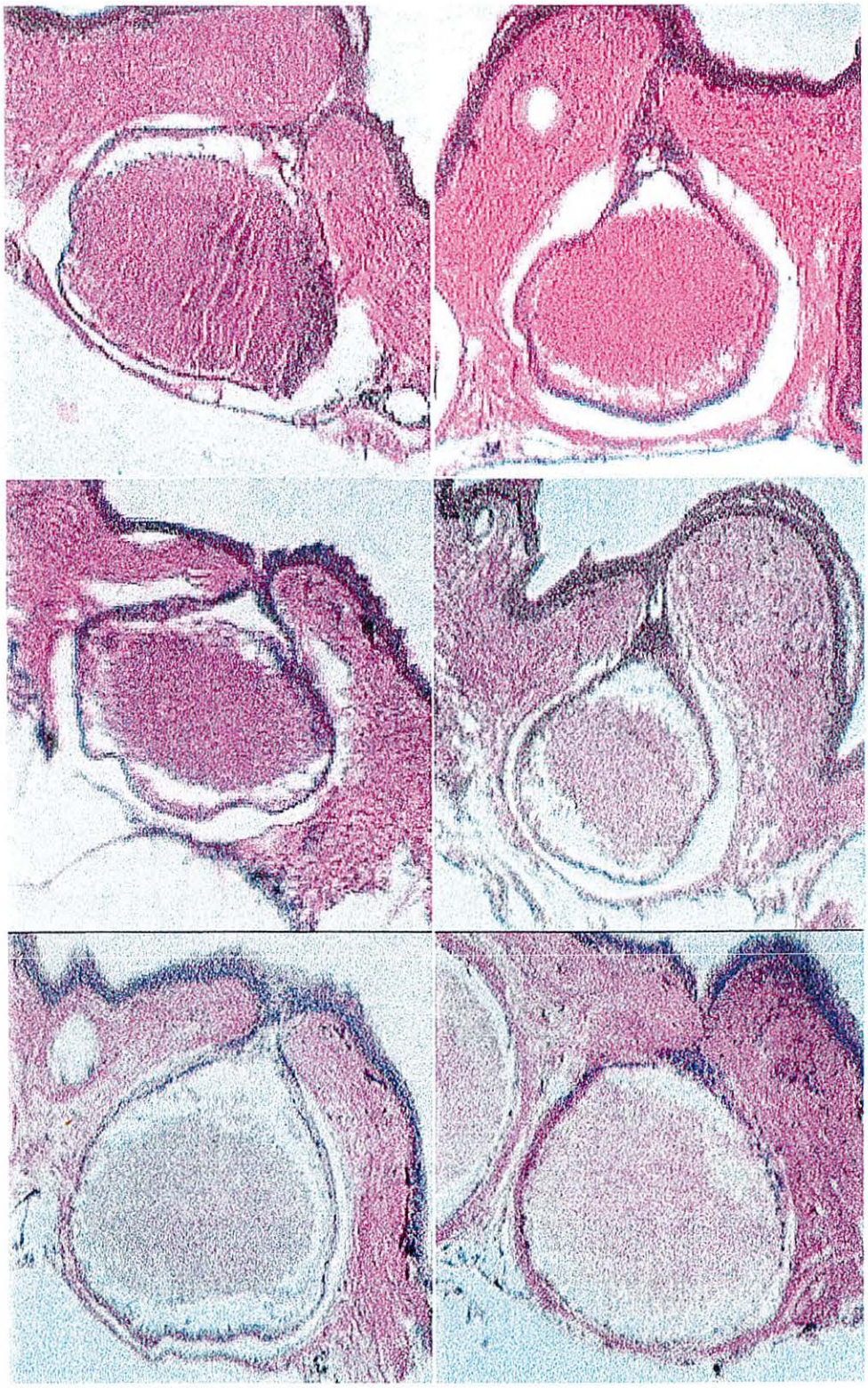


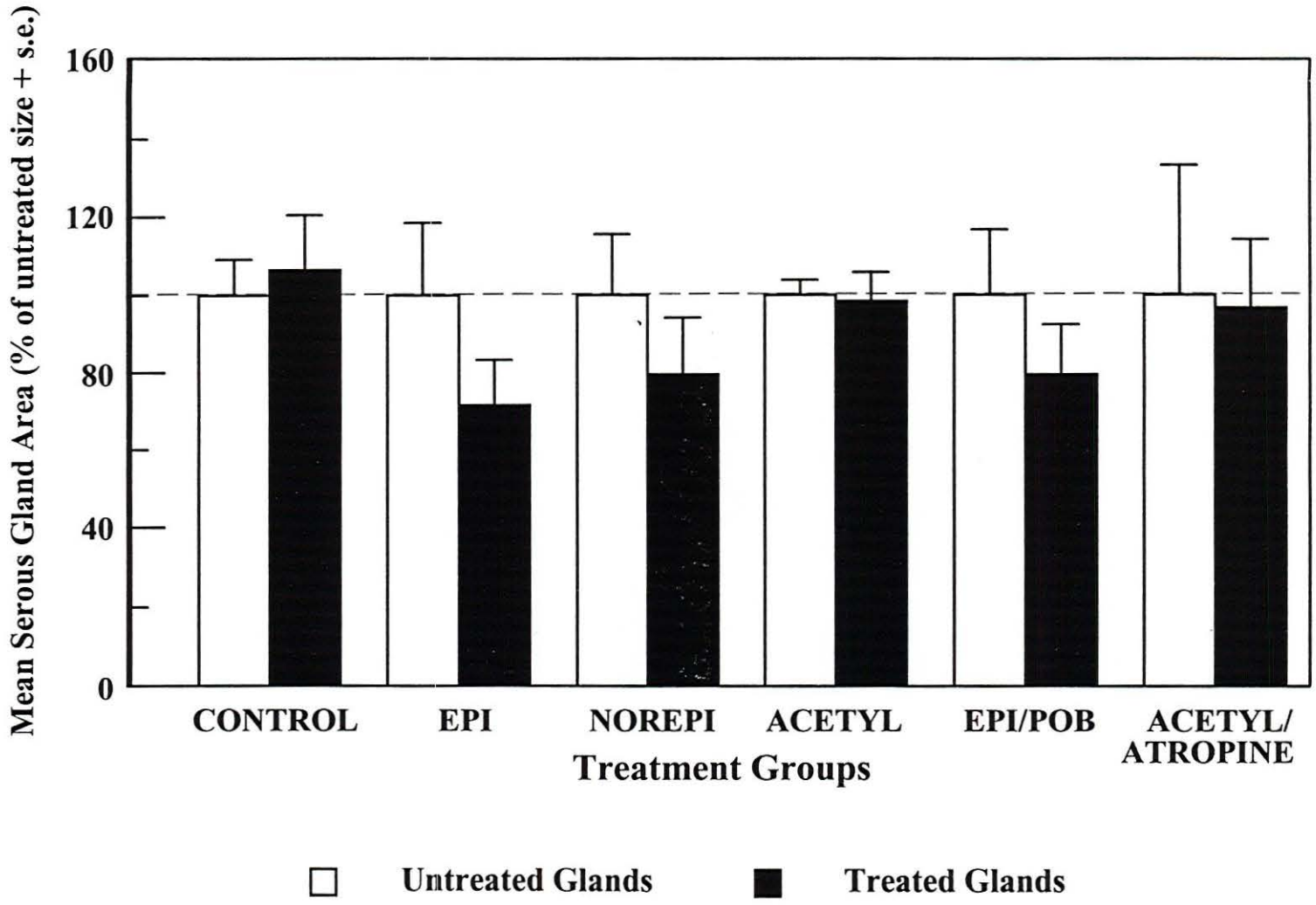
A

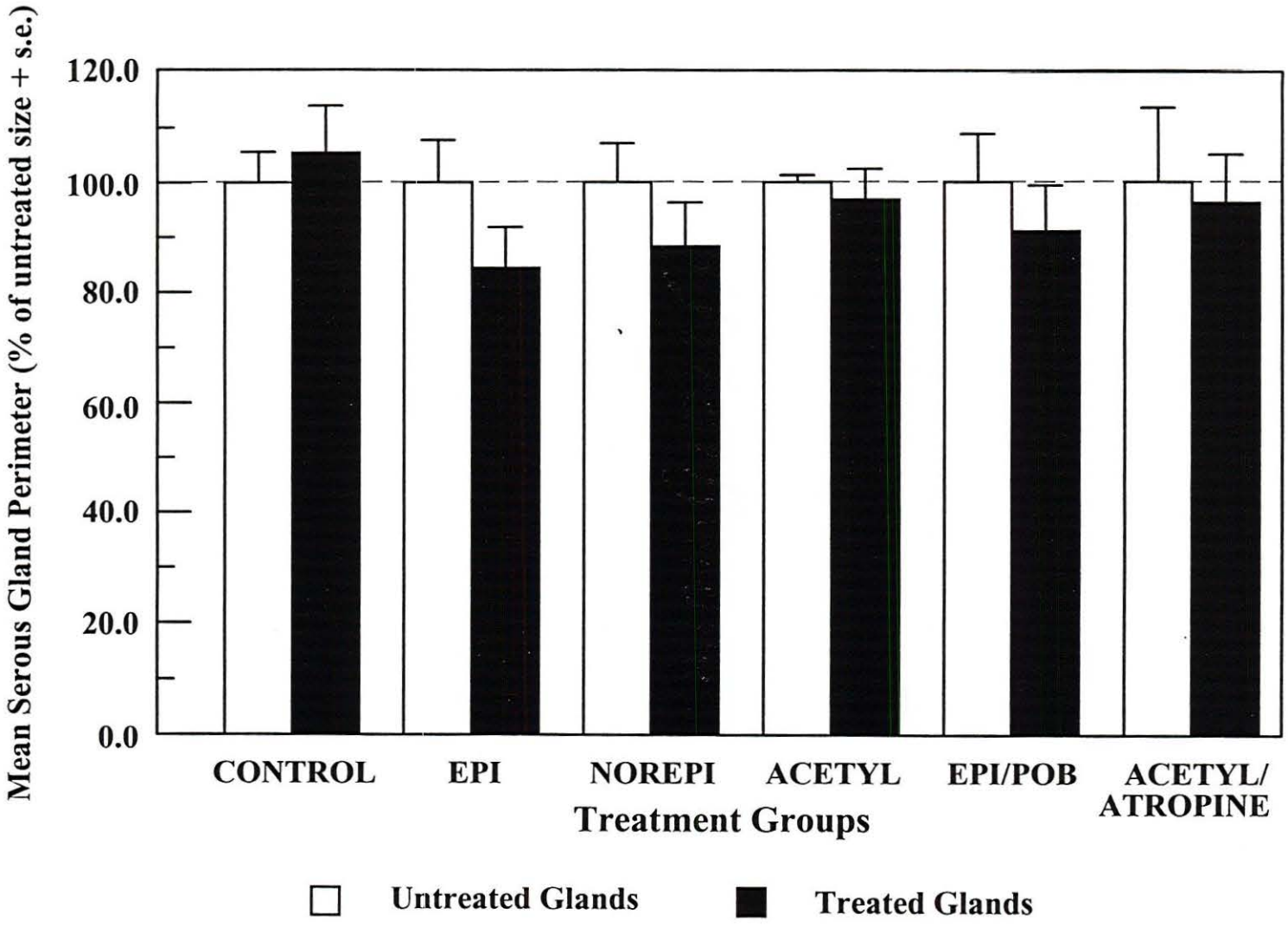
B

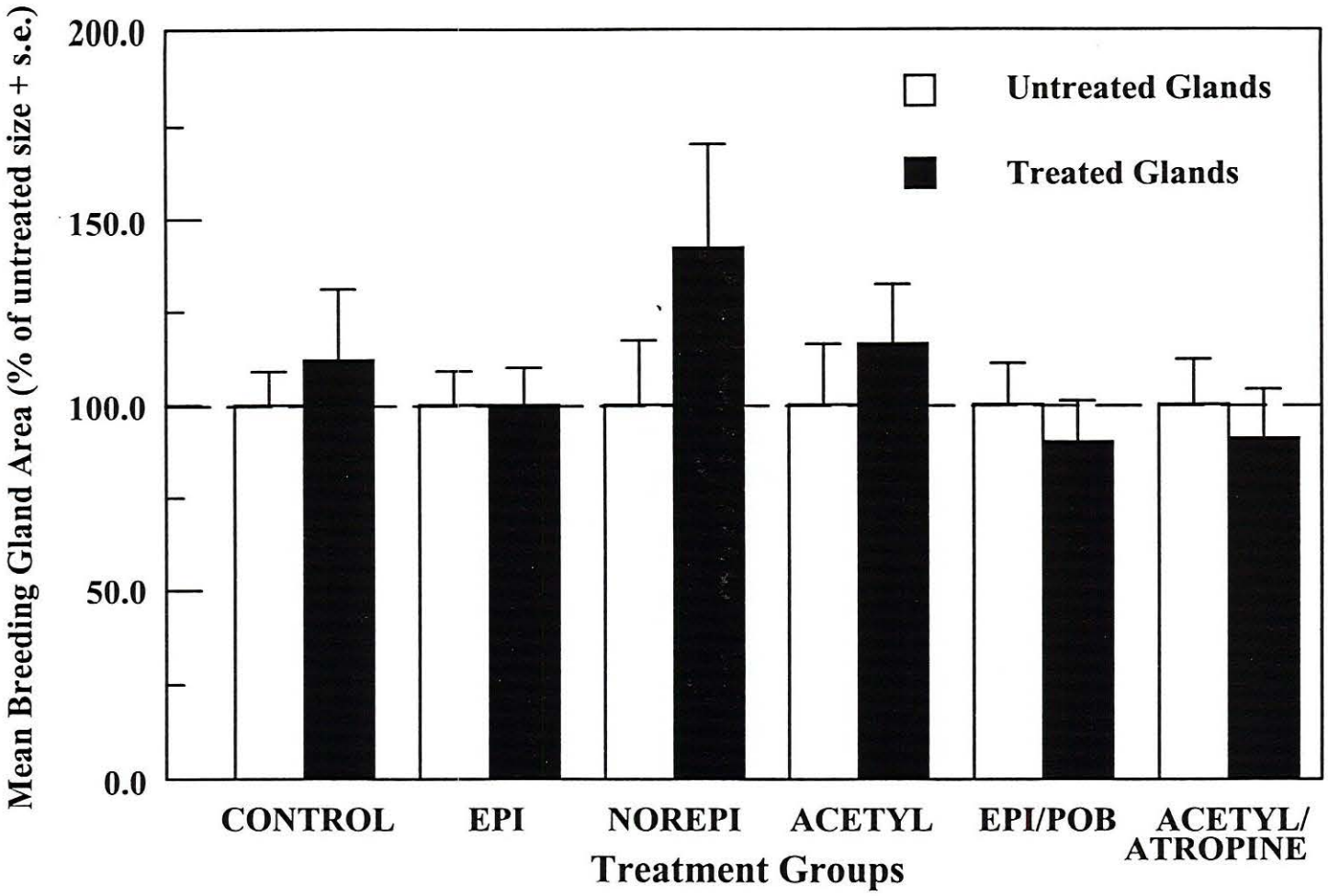
C

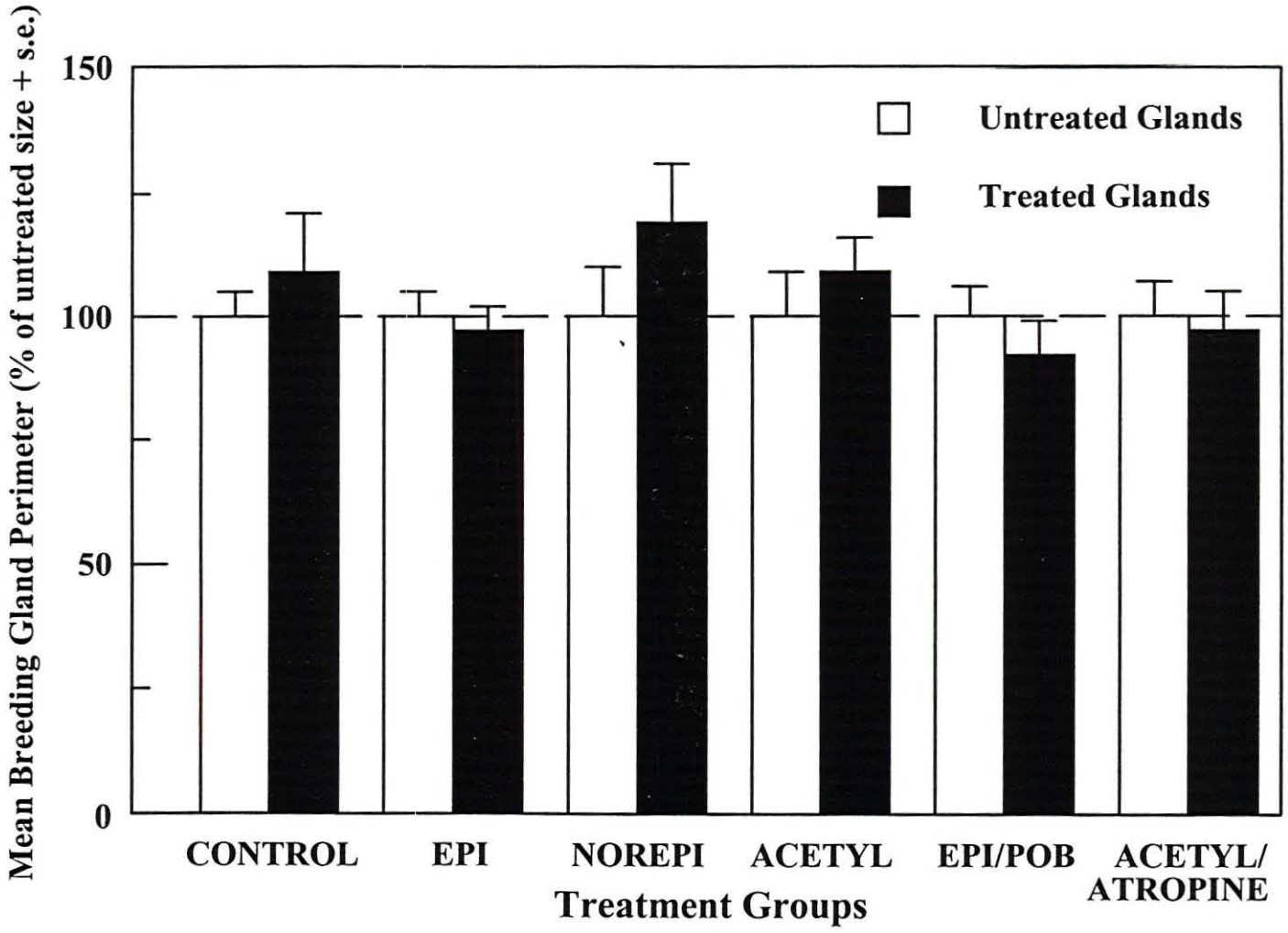
D

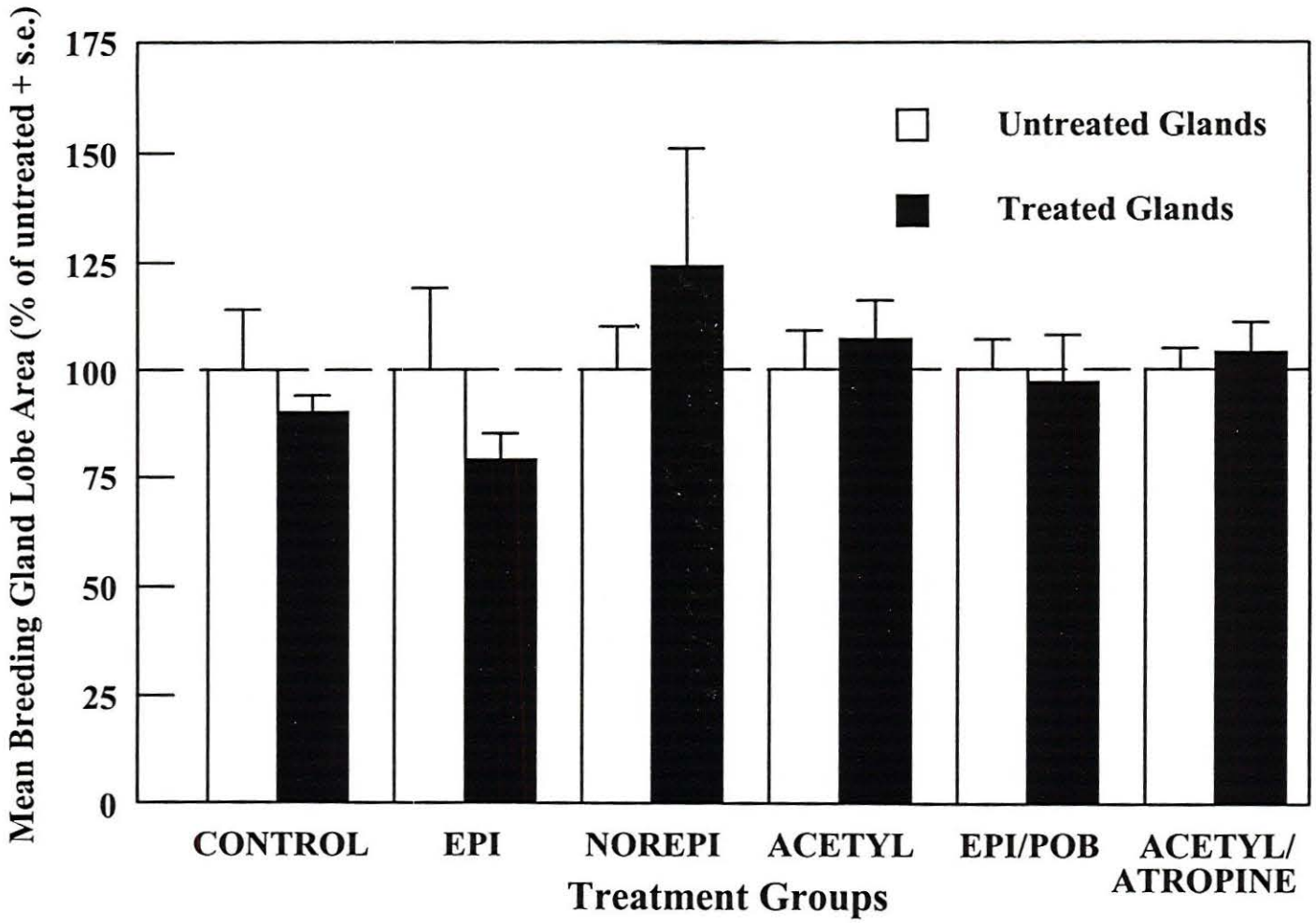

A
B
C
LEFT
RIGHT

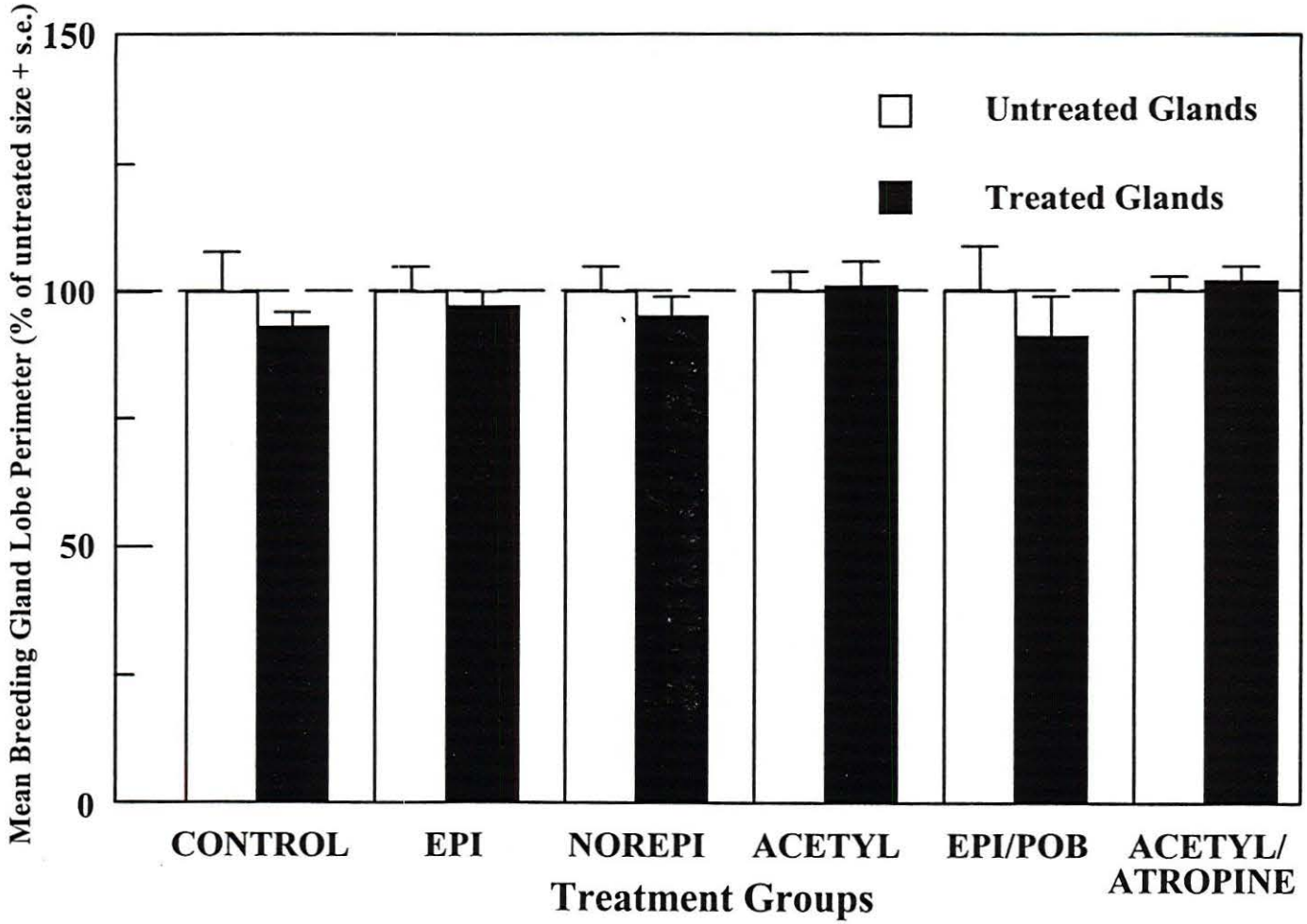

Mean Serous Gland Area (% of untreated size + s.e.)
160
120
80
40
0
CONTROL
EPI
NOREPI
ACETYL
EPI/POB
ACETYL/ ATROPINE
Treatment Groups
Untreated Glands
Treated Glands

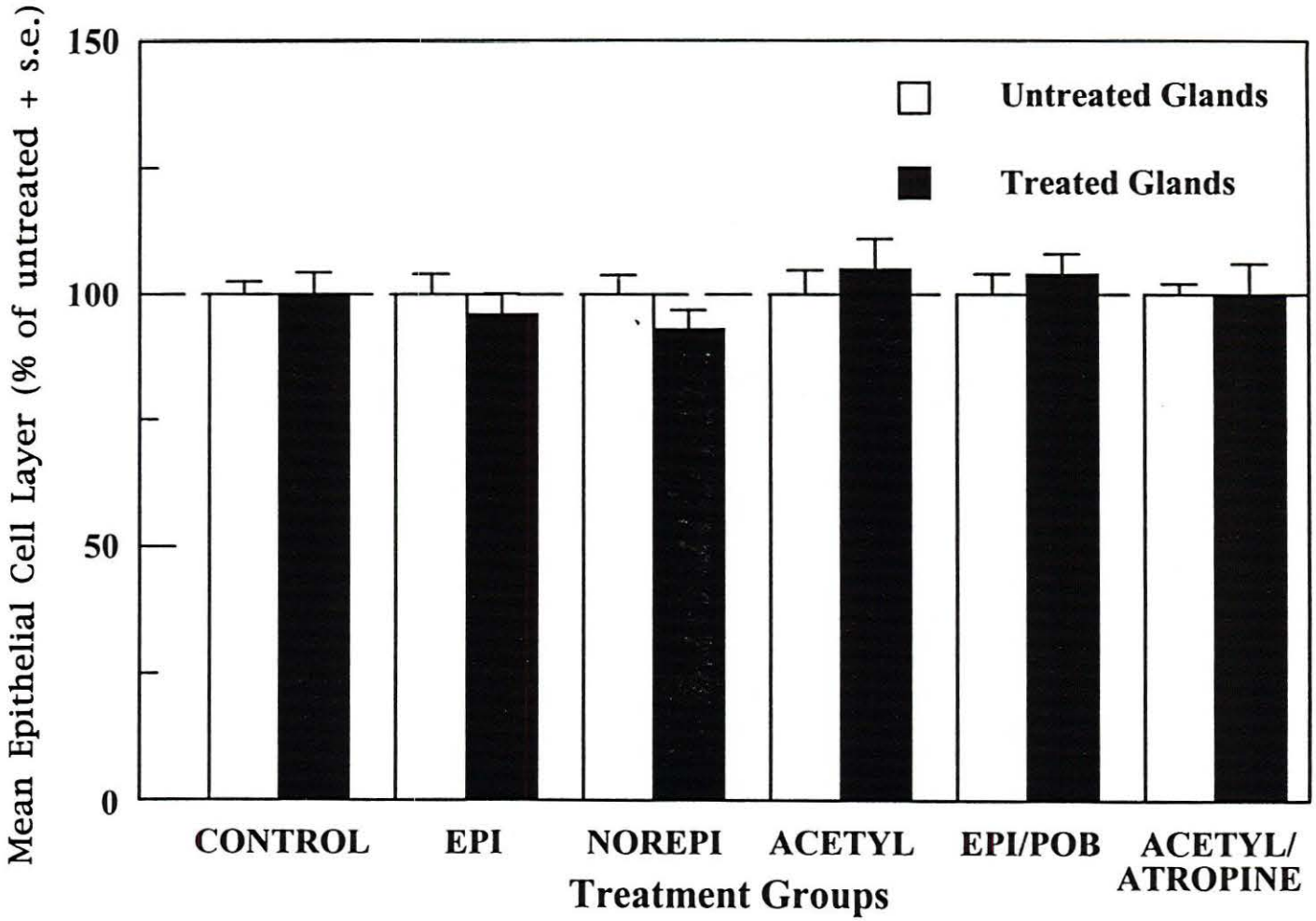

Mean Serous Gland Perimeter (% of untreated size + s.e.)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
CONTROL
EPI
NOREPI
ACETYL
EPI/POB
ACETYL/ ATROPINE
Treatment Groups
Untreated Glands
Treated Glands


Mean Breeding Gland Area (% of untreated size + s.e.)
200.0
150.0
100.0
50.0
0.0
Untreated Glands
Treated Glands
CONTROL
EPI
NOREPI
ACETYL
EPI/POB
ACETYL/ ATROPINE
Treatment Groups


Mean Breeding Gland Perimeter (% of untreated size + s.e.)
150
100
50
0
Untreated Glands
Treated Glands
CONTROL
EPI
NOREPI
ACETYL
EPI/POB
ACETYL/ ATROPINE
Treatment Groups


Mean Breeding Gland Lobe Area (% of untreated + s.e.)
175
150
125
100
75
50
25
0
Untreated Glands
Treated Glands
CONTROL
EPI
NOREPI
ACETYL
EPI/POB
ACETYL/
ATROPINE
Treatment Groups


Mean Breeding Gland Lobe Perimeter (% of untreated size + s.e.)
150
100
50
0
Untreated Glands
Treated Glands
CONTROL
EPI
NOREPI
ACETYL
EPI/POB
ACETYL/ ATROPINE
Treatment Groups


Mean Epithelial Cell Layer (% of untreated + s.e.)
150
100
50
0
Untreated Glands
Treated Glands
CONTROL
EPI
NOREPI
ACETYL
EPI/POB
ACETYL/
ATROPINE
Treatment Groups

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